Human spleen DNA

ag N . ^{32}P 2633 (into the anchor primer)

follow P. 53 except use more ^{32}P ATP

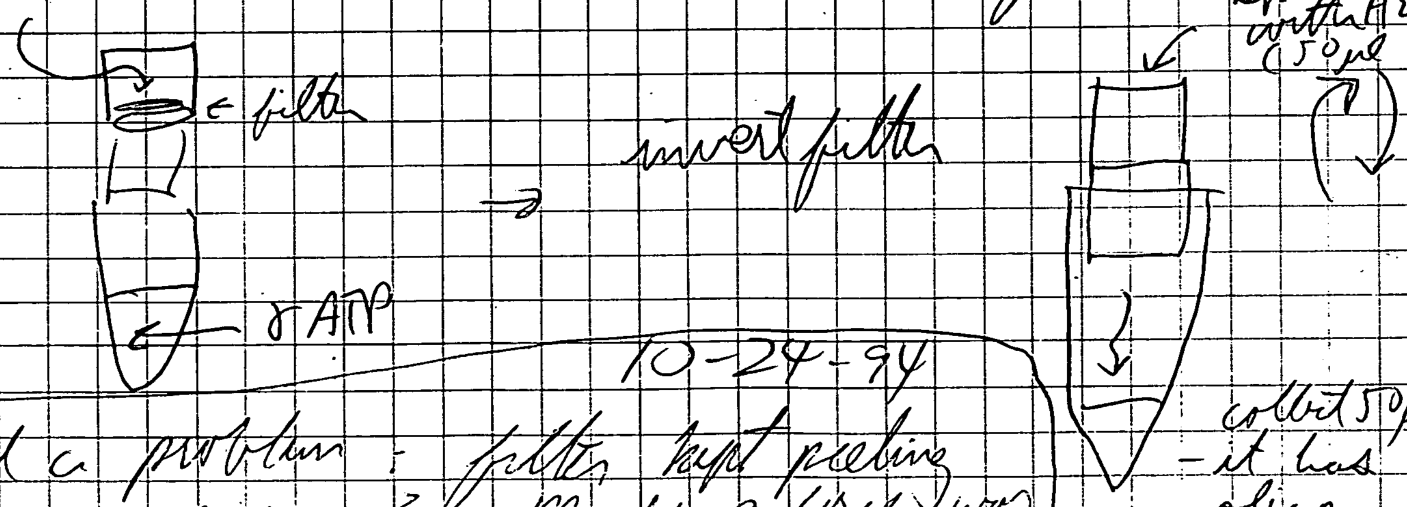
~26% primers now ATP is 100% efficient in labeling

		(1)		(15.9 pm primer) (2)	
oligo 2633	159 µM	1 µl	✓ ✓	✓	dry down
^{32}P γ ATP	6000 Ci/mmol	25 µl	✓ ✓	✓	1 kb ladder
10 mCi/µl 10-21-94			(41.8 pm ATP)		10 µl H_2O
(1.67 µM ATP)					1 µl ^{32}P dCTP
5X Kinase buffer		6.75	✓ ✓	✓	15' 37°C
PNK 50 u/µl		0.25 µl	✓		1 µl EDTA
		33.75			

37°C 30 min → 5' 55°C → add

~~spin col same as p 154,7, and 145,3~~

dilute ^{32}P2633 with 100 µl H_2O (V_f = 133 now)
spin in microfuge in "micron 3"
(amicon # 42403) - after all went in, put
add 200 µl more H_2O and spin again
remove volume that did not enter filter



10-24-94

Had a problem - filter kept peeling
back on micron 3. Maybe g force was
too high on Beckman microfuge "E" model
will skip separation of free ATP.

^{32}P 2633 is diluted only 33.75 fold for
C_f = 4.71 µM

Witnessed & Understood by me, [signature] Date 10/24/94
Invented by [signature] Date 10-19-94
Recorded by 10/24/94

[redacted]

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Project No.______ Book No.______ TITLE 13.5 Kb long PCR

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		①	②	
^{32}P 2633 4.71 μM	✓	4.7 μl	4.7 μl	0.2 μ
2628 cold 199 dilute to 10 μM	✓	2.2 μl	→	0.2 μM
80 ng/μl Human spleen DNA	✓	1.1 μl	→	(80 ng/1
4 dNTPs 10 mM each	✓	2.2 μl	→	200 μM
pol mix: Vent 2u/μl 0.5 μl; Tfl 1u/μl 15 μl; Vf = 15.5 μl →	✓	1.36 μl	4.08	Total (1.32 unit, 0.088 u Vent in ①. 3 more in ② = 0.26 u Vent
5X buffer Cheng	✓	22	→	
$Mg(OAc)_2$ 12 mM	✓	11	→	Cf = 1.2
H_2O	✓ 32.2/[illegible]	~~75.34~~ 65.44	~~72.6~~ 62.7	
		Vf = 110 μl	110 μl	

remove 10 μl to 2 μl 0.2 M EDTA at 0 cycles.
remove 10 μl at 5, 10, 15, 20, 25, 30, 36

program 139 15", 94°C → 20 min, 68°C
140 10', 72°C
141 1', 94°C

142 = 141, 139, 140, 4

started at 8:16 AM
20.5 min/cycle

need 12 hr, 20 min to complete so expect to end at 8:40 – 9:00 pm

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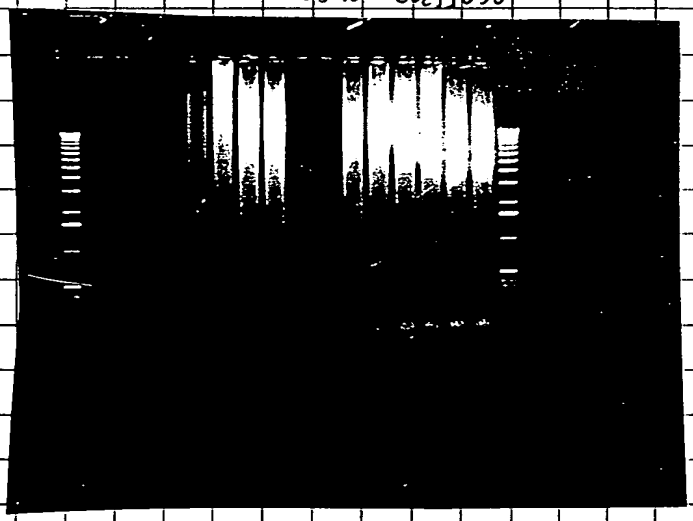
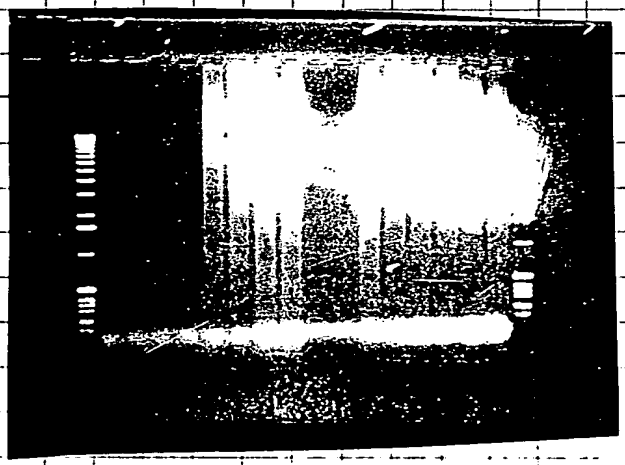
Recorded by

ag N .— 8% agarose same as P.56

Tf1 : 1.33 4

05 10 15 20 25 30 35 05 10 15 20 25 30 35





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